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Research Article

Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda

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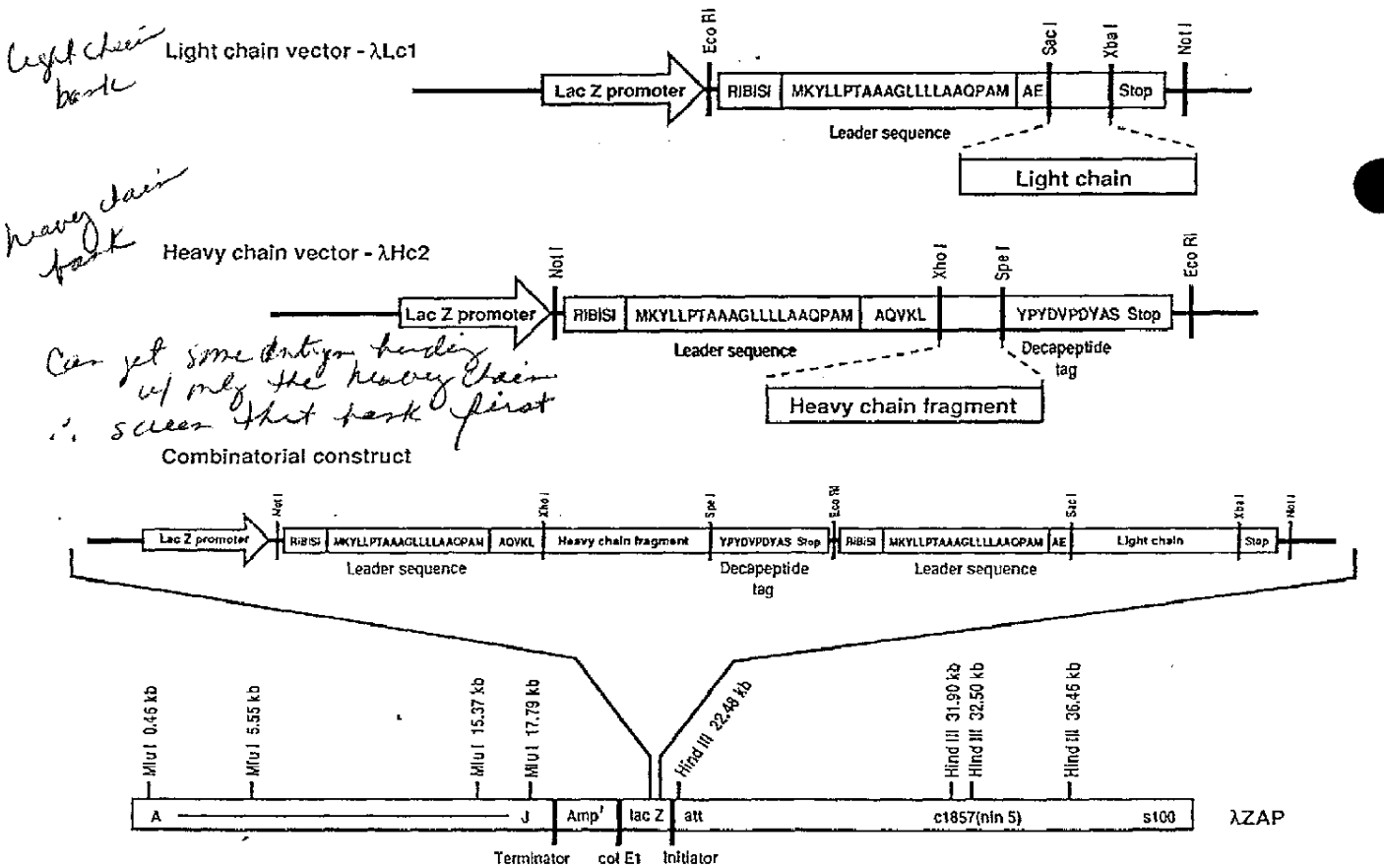

Fig. 1. Combinatorial bacteriophage λ vector system for expression of Fab antibody fragments. The λLc1 vector was constructed for the cloning of PCR amplified products of mRNA that code for light chain protein by inserting the nucleotide sequence shown depicted in Fig. 2A into the Sac I and Xho I sites of λzap II. The sequence was constructed from overlapping synthetic oligonucleotides varying in length from 25 to 50 nucleotides. The λHc2 vector was constructed for cloning PCR amplified products coding for heavy chain Fd sequences by inserting the nucleotide sequence (Fig. 2B) into the Not I and Xho I sites of λzap II. As with the light chain vector, the inserted sequence was constructed from overlapping synthetic oligonucleotides. The combinatorial constructs that can express Fab fragments are generated by cutting DNA isolated from light and heavy chain libraries at the antisymmetric Eco RI site of each vector, followed by re-ligation of the resulting arms. This generates constructs having random combination of light and heavy chains which can be expressed, upon induction with IPTG, from a dicistronic mRNA from the lac Z promoter.

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